ABSTRACT

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Human membrane bound enzymes involved in the metabolism of carbonyl containing compounds consitute a highly interesting group of enzymes. Despite the great effort of scientists over the world most of them still remain uncharacterized, mainly those from the large short-chain dehydrogenases/reductases superfamily (SDR).

At present, 75 SDR members have been indentified in the human genome whereas only 20 % of them is considered to be well characterized. On the other hand 30 % SDRs remain completly uncharacterized. SDR enzymes are involved in the metabolism of steroids, saccharides, retinoids or prostaglandins and therefore play a crucial role in a number of physiological pathways as well as several serious diseases (e.g. hormon dependent cancer, metabolic syndrome, diabetes mellitus). Moreover, SDR proteins contribute to the biotransformation of some xenobiotic compounds. Several SDR enzymes have been identified to be involved in the phase I metabolism of drugs such as doxorubicin, dolasetron, benfluron, metyrapone or ketoprofen, but so far only one membrane-bound member, 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1). With regard to xenobiotics' lipophilicity which affects their distribution within the cell, it can be expected that right membrane-bound reductases play an important role in the biotransformation such substances. Moreover, this hypothesis is supported by the findings that in many cases the stereospecificity of metabolites resulting from biotransformation of prochiral carbonyl group containg componds can not be explained by the activity of only one enzyme.

For better understanding of involvement of membrane-bound reductases in the metabolism of carbonyl compounds, at the first stage *in vitro*, it has been prepared the recombinant forms of more or less known or completly unknown SDR enzymes. The enzymatic activity so far uncharacterized dehydrogenase/reductase (SDR family) member 7 has been detected during the selected substrates screening.

For the first time the computatinal predictions of DHRS7 as transmembrane protein, its topology and basic biochemical properties were experimentaly confirmed. DHRS7 has been demonstrated to be an integral protein with the main part of polypeptid chain facing the lumen of the endoplasmic reticulum with the lack of posttracriptional glycosylation modification. Subsequently, NADP(H) cofactor preference and enzymatic reducing activity of DHRS7 was determinated toward endogenous substrates with steroid structure (cortisone, 4-androstene-3,17-dion, estrone) and also toward relevant exogenous substances bearing the carbonyl group harmful to human health (1,2-naphtoquinone, 9,10-phenantrenequinonechinon, 1,4-benzoquinone). Moreover, the enzyme was succesfully solubilized thanks

to suitable detergent selection, purified and reconstitued to the liposomal system. Due to reconstitution the further detailed studium of DHRS7 is possible.

Focused on carbonyl compounds metabolism the new methods of high performance liquid chromatography were established for detection of reduced metabolites of bupropion, tobacco specific nitrosamin NNK and endogenous all-*trans*-retinal. The enzymes from aldo-ketoreductase and short-chain dehydrogenase/reductase superfamilies were identified in biotransformation of these compounds at least *in vitro*, so information gaps in their metabolic profiles were filled. 11β-HSD1, AKR1C1, AKR1C2, AKR1C3 and carbonyl reductase 1 were determined to take part in the metabolism of bupropion. Newly DHRS7 was identified to contribute to the enzymatic reduction of NNK and all-*trans*-retinal.